Immune system protects integrity of tissues

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Abstract

The immune system neither discriminates between ‘Self’ and Nonself’, nor it acts when confronting ‘Danger’, rather, it reacts to disruption of tissue integrity allowing its renewal. The ‘integrity’ hypothesis proposes three groups of signals that coordinate actions of dendritic cells and immunocytes during the initiation of the specific immune response, and suggests explanations for tolerance, memory formation, and repertoire selection, including differences with other theories. © 2001 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Most of us are aware about the enormous complexity of atmospheric conditions in our environment. Comparing them with immunity is probably useful in just one aspect. If Self is taken as a fair weather and Nonself as a bad one, then the complexity of the system is clearly ignored, even if we define the bad weather as dangerous (and everything that leads to it). For this reason, I wished to add a degree of complexity in the analysis of the immune system.

2. The integrity of tissue

A cell communicates with its environment (intercellular matrix and adjoining cells) via molecules that are either soluble or cell-membrane bound products. Soluble molecules are messengers, while the cell-surface ones can be grouped into two main categories according to their function: physical adhesion and signaling. The signaling molecules can transmit either in-going (receptor) or out-going (sender) type of signals. The adhesion molecules might also have a receptor-like inward-bound signaling function. Since receptors that bind cell-surface or matrix molecules inadvertently contribute to adhesion of a cell, the distinction between the two categories is definitely blurred. The in-going messages from adhesive-molecules and inward-bound and outward-bound signals of proper signaling molecules constitute the ‘integrity’ signals. The integrity of a tissue is, thus, a measure of all-possible adhesive and signaling contributions that a single cell accepts and sends in its normal, resting state.

3. The immune response

The cells of the immune system that survey tissues can be grouped into three major types:
1. the presenters (DC, FDC, MΦ, B and other APC);
2. the responders (naive and memory immunocytes, NK, γδT); and
3. the effectors (eT, eB, plasma, eNK, eγδT).

The signals that regulate the workings of the immune system depend on three groups of signals, called the sender (signal-1), the modulator (signal-2) and the alert signal (signal-3). All three signals produce a qualitative difference in cellular function by quantitatively altering the composition of intracellular mediator molecules. They may have a variable time-frame of action, because they can induce either fast changes in cytoplasmatic mediators as well as delayed changes by induction of de novo protein synthesis. The functions like the initiation of the immune response, infected-cell elimination, antibody production, other effector functions, memory cell formation, locomotion and the development of im-
munocytes can be explained with the three-signal system. The restoration (regeneration) of a tissue is the function of a tissue itself. The explanation of some functions will be used as examples in describing the signals.

1. The sender signal (signal-1) comprises a group of various intracellular signals that are necessary and sufficient for the activation of effector cells (Fig. 1A). However, signal-1 is not sufficient for the activation of responder cells (see signal-2), where it can lead to programmed cell death, provided the cell is in an alert state (see signal-3). Signal-1 can be a group of inward-bound intracellular signals in immunocytes. It results in formation of intracellular, extracellular and transmembrane molecules that are capable of further in- or out-going signal transmission. Signal-1, for example, includes a communication between the dendritic cell and the responder T cell via TCR, coreceptor and peptide/MHC molecules; or antigen binding to BCR on responder B cells.

2. Signal-2 consists of a group of intracellular signals that modulate signal-1 (Fig. 1B). There are two modes of action regarding signal-2: positive and negative mode. In the positive mode, it can rescue cells from apoptosis induced by signal-1 (negatively regulating parts of signal-1 by, perhaps, downregulation of the mediators of apoptosis). Positive signal-2 can fix DNA accessibility and skew the levels of second messengers induced by signal-1. In its negative mode, it can potentiate signal-1, and even lead to a faster cell death. For T cells, signal-2 can be a group of inward-bound signals like, for example, costimulatory (B7/CD28 in a positive mode; or B7/CD152 in a negative mode). For a myeloid DC, a second signal would include ‘crosstalk’ between DC and the responder T cell (in a positive mode) enhancing DC antigen presentation during maturation of DC. For a B cell, it includes T-cell help in a positive mode. However, a negative signal-2 for a B cell would be a regulatory T cell.

3. Signal-3 controls the access to DNA in the nucleus, which would include chromatin reorganization. Because of this, signal-3 can modulate signal-1 and signal-2. Like the first two signals, signal-3 is different for different cell types. For immunocytes, it comprises a complex network of signals initiated by cytokines, hormones, chemokines, cell–cell, cell–matrix, and other non-protein signals that can function as a ‘gate’ to other cellular functions (Fig. 1C). For myeloid DC, signal-3 is one that results from disruption of homeostatic signals. The latter I call integrity signals of a tissue. The break up of inward-bound integrity signals, as in usual tissue disruptions due to infection, inflammation or physical damage, alerts dendritic cells (i.e. they become ‘mature DC’) to initiate the immune response (Fig. 2). For example, signal-3 can upregulate costimulatory molecules CD80/86, which provide the signal-2 for T cells. It can also modulate signal-1, perhaps, by altering the conformation of peptides presented on MHC molecules in DCs (Fig. 2). The variability of integrity signals in tissues is large. For ease of understanding, they can be compared with colors. Thus, each signal-3 can have a different color. It implies that signal-3 would facilitate differentiation of specific immunocytes into different classes of effector cells. For example, such ‘alerted’ T-cell effectors can help or kill immediately upon the receipt of signal-1.

The activation of specific immunocytes is defined as capacitation of T or B cells to eliminate or suppress a pathogen (or parasite, in a wider meaning). The activation of the immunocytes would include peripheral maturation from the naive or memory cell into a number of effector states and classes, concomitant proliferation, induction or change in the expression of various receptors, membrane-bound or cellular proteins and secretion of various products including cytokines and chemokines. The initiation of the immune response is defined as the activation of naive immunocytes. The signal-3 could be mistaken for the first step of the activation. It should be distinguished from it, as it may be the first stage of the apoptosis, too.

4. The class of the immune response

Effector T cells can be of Th0, Th1, Th2, Th3, Tr, Tc1 and Tc2 type (so far). These types of cells produce qualitatively different immune responses, defining the class of the response. The formation of different classes of the response would be controlled by a distinct combination of three signals that a responder immunocyte receives, especially regarding the ‘color’ of signal-3. Immunocytes of different classes would differ in DNA accessibility for factors that regulate class-specific genes like those encoding various cytokines, chemokines and...
their receptors. For example, the activation of T cells is influenced by the color of signal-3 (the cytokines in environment) that leads to Th1 or Th2 response. For B cells, different colors would induce Ig class switching and lead to Ig isotype differences.

5. Anergy and memory

The effectors are cells that by definition have signal-3 ‘on’. In other words, they have a terminally-induced differentiation state whereupon they can live for a short period. If they get signal-1, the effectors will die faster, but not before the execution of their function. However, if there is a shut-off of signal-3 during or after the initiation of the immune response, the result will be a resting state, in which cells cannot respond to signal-1 and signal-2. Let us consider two examples:

1. The first is when the shut-off of signal-3 breaks signals-(1 + 2). This can happen at earlier or later stages of (1 + 2) signaling. As a result, T cell becomes blocked or anergic, and will live until mechanically destroyed, or by age and other signals. Later on, if the third signal is delivered again (because neither signal-1 nor signal-2 can yield reactivation) the cell will die by apoptosis as incomplete signal-2 would not suffice for rescuing. Rarely, the T or B cell can become reactivated, if previous signaling (1 + 2) was near completion, and the same color of the signal-3 was provided again. Signal-3 can be switched off due to establishment of ‘normal’ integrative signals of a tissue, or via other mediators in special circumstances (tumor).

2. The second consequence is when the signaling (1 + 2) is complete and when immunocytes have become effectors. To function as effectors, they would need the signal-3 on, but because it is being shut-off, they become memory cells. The shutting-off of signal-3 cannot completely reverse their DNA accessibility to an earlier stage (naïve T or virgin B cell). The experience of past activation is represented as a ‘scar’ at the DNA level, because signal-3 has already fixed the events during the first encounter with an antigen. This makes the memory cells respond faster in the second encounter with signals-1 + 2. Furthermore, if only signal-3 influences a resting memory cell, the memory cell could just divide, without becoming the effector. It follows that memory cell maintenance would not need signal-1. For example, MHC would not be needed for the maintenance of T-cell memory, but it would be required for the establishment of memory cells, and the activation of a memory cell to become an effector again.

6. The evolutionary aspects

I suggest that the mechanism that generates V(D)J rearrangements in TCR and Ig molecules, tries to neutralize all germ-line encoded diversity. The result of the scrambling of the germ-line diversity produces completely new binding properties and, thus, specificity. The recognition function seems to be re-generated for every new member of a species.

In a biological system, the conservation of molecular structure during evolution implies an important function. For example, similarity of cytochrome c molecules among many species points to its non-adaptable and non-replaceable function in the generation of cellular energy. Where do we find such conserved elements in the immune system? The conservation of constant parts of Igs, TCRs and MHC molecules, intracellular mediators, and other cell-membrane molecules involved in immunological signalling, indicates a selective pressure for all three signalling functions except the recognition function (of signal-1) within the immune system. However, considering the V regions of Igs and TCRs, we see a paradox. The germ-line encoded Vs are also conserved. In other words, all but the last antigen-(pMHC-)binding region, encoded by the V(D)J portions in mature immunocytes, are preserved. Thus, we find that immune repertoires may differ in:

1. the usage of clonally distributed germ-line V region alleles (including Ds and Js);
2. somatic V(D)J rearrangements during development of T and B cells; and
3. somatic selection of individually distributed repertoires (positive and negative selection).

This variability in individual repertoires may, at a first glance, mean an indifference in evolutionary terms for the binding specificities of the recognition structures in the signal-1. However, we see that the framework and the first two/three antigen-(pMHC)-binding sites of the V regions are evolutionarily conserved. I suggest that this is so because of a need for internal communication, in particular, between the inducing/responder and the effector/target branch of the immune response. The pressure acting on maintenance of the ‘interactivity’ within the immune system would predict the finding of ‘mutually interactive’ sets of TCR/MHC and Id/anti-

Id (or Ig?) combinations within the ‘non-selected’ repertoires of related species, declining with evolutionary distance. Next, this interactivity renders any defense system vulnerable to pathogens that can change its dominant antigenic profile and produce an imitation of Self. To solve this problem, organisms evolved that could scramble the original interactivity, and re-assemble it with each new generation. Thus, in the primordial immune system, the uniform MHC-binding receptor may have become scrambled (rearranged), and upon re-assembly, each new variant of V, that helped in
fighting off intruders, would have been selected to stay in the germ-line but only if it was also interactive. It follows that the diversity of recognition is evolutionarily selected by two opposing selective forces:
1. a pressure to bind Self for communication (preserving TCR/MHC and Ig/\textsuperscript{\textalpha} or Ig-Id/Ig-anti-Id combinations); and
2. a pressure not to harm Self (tolerance) by conserving molecules involved in scrambling the evolutionarily selected (parts of) molecules selected by the first force, namely, the V regions of Igs and TCRs (gene rearrangement) or MHC alleles (gene conversion).

The second force can effectively destroy all the binding that is conserved by the first force in just a single 12 generation: V paratopes and alpha helices of MHC molecules. This force (‘not to harm S’) may be confused with, and assigned as the selection for binding NS! On the contrary, in order to re-establish effective communication within the immune system, a repertoire of recognition has to be tailor-made for every individual during ontogeny. This correlates to positive selection of developing T and B cells.

In conclusion, the specificity of the immune response or the variability of structures involved in signal-1 have an evolutionary advantage over uniform defense mechanisms. Specific recognition of antigens by B and T cells is a part of the evolutionary process that aims to protect the integrity of tissues through cellular intercommunication. For immunocytes, it was advantageous to more precisely link afferent and efferent arms of a defense system. The ‘integrity’ hypothesis proposes that the survival of the ‘integrity of a tissue’ within an organ depends on the immune system. It reacts to a disruption of tissues and allows renewal. The repertoire of immunocytes represents a data base of past disturbances and intelligence network preventing tissue dilapidation.

7. The predictions

All three types of cells involved in the immune response can be controlled with the sender, modulator and alertness signals. The challenge would be to identify molecules that fit these descriptions in DCs, B, T, and NK cells. Alerted DCs might provide a different ‘color’ of signal-3 to responding immunocytes. Some might ask, why do we need theories, and groups of chaotic signals, when we will relatively soon know all intracellular mediators in the coming post-genomic era. Well, it is just another human characteristic to make life more interesting, colorful and challenging. For example, which mediators are more important than the others? In other words, which ones shall we pursue first (and try to use inhibitors in clinics)? Good theories give major advantages. I’d like to call such theories — catalysts of cultural progress.

Signal-3 represents a complex group of signals. Some of those can modify signal-1 at various points. Signal-3 might upregulate enzymes that affect post-translational modification or processing of (Self and Non-self) antigens. The presentation of an antigen would not be modified, because this change might only slightly alter the conformation of the peptide. Therefore, a peptide might still bind the MHC molecule, but it would provide a different T-cell epitope. For example, this could be achieved by deamination of lysines in peptides, which introduces a charge difference, similar to deamidated gliadin peptides that can induce coeliac disease (Molberg et al., 1998). It follows that constitutively active individual components of signal-3 could form a basis for the immune-cell attack.

The complexity of signal-3 is defined as all possible combinations of mediators that will modify signal-2. In principle, signal-3 will upregulate (positively) signal-2 to provide co-stimulation for B or T cells. Thus, co-stimulation for a B cell would be not only T-cell help, but also cross-linking of various B-cell membrane components that would yield similar intracellular transmissions, perhaps similar to suggested T-independent B-cell activation upon cross-linking of LPS/Toll receptors (Moller, 1999).

Signal-3 can be possibly switched off due to establishment of ‘normal’ integrative signals of a tissue, or via other putative mediators (mimicry of integrity?). It is possible that there is a refractory period until T or B cells can be alerted again. The refractory state could be different for various cells and states.

The classes of immune responses are generated by the differences in the ‘color’ of signal-3 delivered to T cells.

Memory cells can be generated by shutting-off of the signal-3 in effector cells.

Tolerance can be achieved by the delivery of signal-1 (clonal deletion), or signal-1 in combination with the negative-mode of signal-2 to immunocytes in alerted state. The classes of immune response that generate Th1, Th2 or Tc1 and Tc2 are generated by different ‘colors’ of signal 3 delivered to T cells upon activation by signal-1 and positive signal-2. However, the same colors can produce different classes, if signal-2 were negative (CD154) resulting in, for example, regulatory Tr1 and Th3 cells instead of Th1 and Th2. Such cells could compete or inhibit other classes of the immune response.

The B-cell receptors (BCRs) might have a corresponding positive-selecting molecule, analogous to the TCR-selecting MHC molecules, which may have been lost during evolution, perhaps due to anti-Id stepping into its place.

Lastly, the immune system could be manipulated to adopt Nonself as Self (and vice versa) by appropriate
modulation of signal-2 and signal-3. This is a testable challenge, and if accepted, it would widen the field of research, in order to find successful treatments for preventing graft rejections and auto-immune diseases.

8. The differences with other theories

The Self–Nonself discrimination principle describes in simple terms how selective rather than instructive mechanisms might operate within the immune system (Lederberg, 1959). Bretscher and Cohn (1970) proposed a model of lymphocyte activation that involves a two-signal activation mechanism, which revolutionized our concept about the initiation of the immune response. Then, Langman and Cohn (1996), in the ‘Associative Antigen Recognition’ model, developed further the S-NS discrimination concept involving the NS-recognition-signal-dependent activation of specific immunocytes. In contrast, Coutinho and Stewart (1991) and Bandeira (1996) proposed a dominant suppression concept based on Jernean-idiotype network and S/NS discrimination principle (‘Suppression network’). Next, Janeway (1989) in ‘Stranger’ and Fuchs and Matzinger in ‘Danger’ (Matzinger, 1994; Fuchs and Matzinger, 1996) models, assigned an alarming signal for the initiation of the immune response upon ‘intrusion of the pathogen’ (stranger; infectious-Self) or when ‘cellular (or tissue) death (necrosis), distress and disruption’ (danger) are sensed. There are other theories like ‘Morphostasis’, ‘Cytokine-burst’ or ‘Antigen localization’ (Ignorance), by Cunliffe (1995), Weigle (1995) and Zinkernagel (1996), respectively, which stressed the importance of the context of an antigenic challenge. The ‘Integrity’ model formulates a novel set of rules that might operate within the immune system, and was first mentioned in 1996 (Dembic, 1996) and discussed on the internet in 1997 (Bandeira et al., 1997).

Here are the differences among the various theories about the workings of the immune system from the point-of-view of ‘Integrity’.

The Self–Nonself discrimination principle means that signal-1 and signal-2 (without signal-3) suffice for the initiation of the immune response (Langman and Cohn, 1996), whereas, all three signals are required in ‘Danger’, ‘Stranger’ and ‘Integrity’ theories.

The ‘Danger’ (Matzinger, 1994; Fuchs and Matzinger, 1996) and ‘Stranger’ (Janeway, 1989) theories differ from ‘Integrity’ by proposing that signal-3-like events are positive inward-bound signals for DCs in the initiation of the immune response. ‘Danger’ implies that the recognition of some cellular subparts is sufficient. However, ‘Stranger’ claims that a recognition of some kind of general property(ies) of intruding microorganisms (infectious-self) is the initiator. ‘Integrity’ proposes that a break-up of constant networking signals of cells within a tissue would be recognized as signal-3 by DCs (Fig. 2). Furthermore, there is no mentioning of signal-3 (or access to DNA) for immunocytes playing any role in the initiation of the immune response by ‘Danger’ and ‘Stranger’. Similarly, in explaining development, memory and class of the response, ‘Danger’ and ‘Stranger’ theories do not involve signal-3, while ‘Integrity’ does.

The ‘Ignorance’ (Zinkernagel, 1996) hypothesis proposes that immunocytes are localized in lymph nodes until a dendritic cell arrives and provides two signals for the initiation of the immune response. As effectors, they may move to the site of an infection. Outside these ‘working hours’, like, for example, when naive or memory immunocytes are by chance in tissues, they cannot receive either signal-1 or signal-2, because they are ignorant. I have two major criticisms for the ‘Ignorance’ theory. First of all, immunocytes cannot be ignorant, because they learned about these signals during ontogeny (positive selection). Secondly, there is a problem with who holds the master switch when ‘ignorant’ cells should become ‘erudite’. Being simply in the lymph nodes does not sufficiently explain this decision. On the other hand, ‘Integrity’ and ‘Danger’ suggests that passing immuno-surveillant lymphocytes in tissues can respond to both signal-1 and signal-2. The reason why they do not become activated is that, in well-integrated tissues, no signal-2 is available. Thus they can only receive signal-1, and be deleted.

The ‘Suppressive network’ (Coutinho and Stewart, 1991; Bandeira, 1996) theory would imply a break of the suppressing interaction of an Ig-Id with its anti-Id as a shift in regulatory forces that would kick start the response. For T cells, it suggests that a loss of a constant, mutually-suppressive interaction between ‘Id’-TCR and anti-Id (pMHC) on APC would provoke T-cell activation. In other words, the initiation of an immune response to an antigen would be the result of a break in the constant signaling (signal-1 + signal-2) interaction within the immune system. In a way, it would be a shift of the reactivity (to NS) rather than the initiation. The ‘Morphostasis’ theory (Cunliffe, 1995, 1997, 1999) has a similarity with Integrity, in claiming that disorganization in tissues provokes the immune response. The ‘Cytokine-burst’ hypothesis (Weigle, 1995) describes a powerful controlling network of soluble mediators. The differences in cytokine quantities could alter the states of immunocytes and cause them to start the response. The ‘Integrity’ theory sees them as not so powerful, but just as the soluble parts of ‘integrity’ signals providing colorful immune responses.

References


